

Use Of The Light Microscope

Name: _____

Date: _____

Period: _____

Possibly the most important instrument that aids a biologist is the microscope. A microscope allows scientists to investigate worlds that are otherwise too small to be seen. An opportunity to learn about and use this valuable instrument is now yours. A light microscope magnifies objects up to approximately 400 times their actual size.

Two types of slides are used with the microscope: prepared slides and temporary wet mounts. Prepared slides are permanent and are made to last a long time. The school usually purchases these slides. Most of the slides you will use in this course will be wet mounted. You will make these slides yourself. As the name temporary wet mount suggests, these slides are not permanent.

In this investigation, you will

- Practice proper handling of the light microscope.
- Learn the names and functions of the light microscope parts.
- Acquire skill in using the light microscope by carefully following all directions.
- Prepare a wet mount slide
- Locate objects under low, medium, and high power magnification
- Learn what the position of an object is when viewed through a light microscope in relation to its position on the microscope stage.
- Adjust the diaphragm correctly to achieve proper light under low, medium, and high power.
- Learn to locate objects at various places in the “depth of field.”
- Use stains to aid in the viewing of objects.
- Compare the area of view under low, medium, and high power magnification.

Materials

Light microscope
Tweezers
Scissors
Magazine page
Cotton ball

microscope slide
water
Thread prepared slide
potato
razor blade (single-edge)

coverslip
dropper
iodine stain

Part A. Learning Microscope Parts and Functions

Procedure

Figure 1

- Look at figure 1. Note that the student is carrying the microscope with two hands. Also note that the microscope is carried straight up. Do not tilt or tip the microscope as you carry it with both hands close to the body. To carry the microscope hold the arm of the microscope in one hand, and place the other under the base for support.
- Look at figure 2. Use the diagram that to locate your microscope parts.

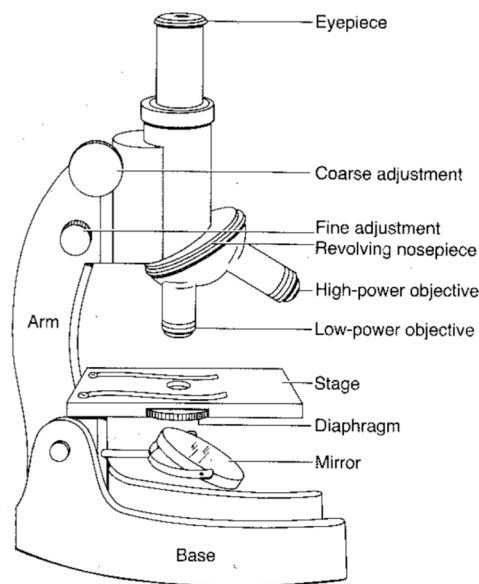
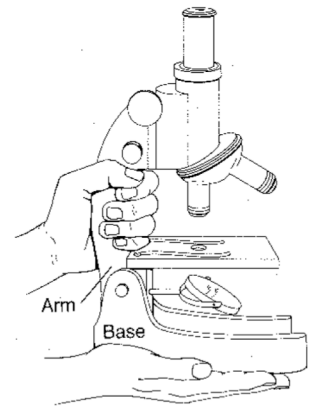


Figure 2

- Following is a list of the parts of the microscope and their functions. Learn the name and function of each part so that you can properly use the microscope.
- *Eyepiece*: The part of the microscope that you look through to view an object placed on the stage. The eyepiece contains the ocular lens and usually has a magnification power of 10X. This means that the lens enables you to see objects ten times larger than it actually is.
- *Objective lens*: Adds to the magnification of the object you are viewing in combination with the eyepiece. The low-power objective lens has a magnification power of 4X. The medium-power objective has a magnification of 10X. The high-power objective has a magnification of 40X. To find the total magnification power of the microscope, multiply the magnification power of the eyepiece lens by the magnification power of the objective in use. For example: eyepiece (10X) X low-power lens (4X) = total magnification (40X).
- *Revolving nosepiece*: Holds the low-, medium-, and high-power objective lenses and can be rotated to change magnification. As you rotate the nosepiece, listen closely to hear the objective lens “click” into place.

- *Stage*: Supports the slide being viewed, and the stage clips hold the slide in place on the stage. In the middle of the stage is an opening that allows light from below to pass through the object being viewed.
- *Diaphragm*: Located under the stage and has several small openings of various sizes. Some microscopes have a lever that can be moved back and forth to change the size of the opening. By turning the diaphragm, you can increase or decrease the amount of light entering through the stage opening to see the object you are viewing more clearly.
- *Coarse adjustment knob*: Moves the eyepiece or stage up and down to bring the object into view. It will be located on the top part of the arm or located near the base of the microscope. Turn the knob and you should notice the eyepiece or stage move up and down. **CAUTION**: *Never use the coarse adjustment knob under high power.*
- *Fine adjustment knob*: Located below the coarse adjustment knob or on the coarse adjustment knob. The fine adjustment knob is used to bring objects into clear, sharp focus. It moves the eyepiece or stage very slightly. Use this knob when you are focusing with the high-power lens.

Observations and Conclusions

1. What are the magnification powers of each of your objectives?

2. What is the highest magnification power of your *microscope*?

3. How should you carry a microscope?

4. Match the microscope parts listed in Column I with their correct function in Column II. Place your answers along the left side of Column I.

Column I	Column II
_____ diaphragm	(a) allows light to pass through stage
_____ stage opening	(b) brings objects into view
_____ eyepiece	(c) regulates amount of light entering scope
_____ low-power objective	(d) is attached to the revolving nosepiece and contains a lens capable of 4X magnification
_____ high-power objective	(e) supports slide
_____ coarse adjustment knob	(f) turns to change from one power to another
_____ fine adjustment knob	(g) contains a lens capable of 10X magnification
_____ stage	(h) brings objects into clear, sharp focus

_____ revolving nosepiece

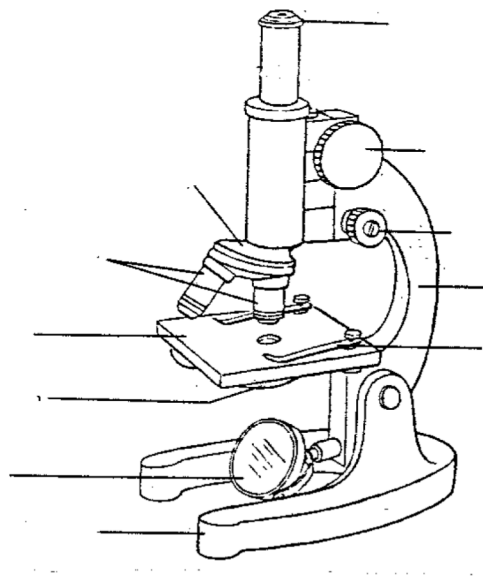
(i) is attached to revolving nose piece and contains a lens capable of 40X magnification

5. Fill in the blanks with the total magnification for each pair of lenses.

<i>Eyepiece</i>	<i>Objective lens</i>	<i>Total Magnification</i>
a) 5X	10X	_____
b) 10X	10X	_____
c) 10X	43X	_____
d) 20X	10X	_____
e) 20X	50X	_____

6. Label the parts of the microscope in figure 3. Check your answers with figure 2-2.

Figure 3



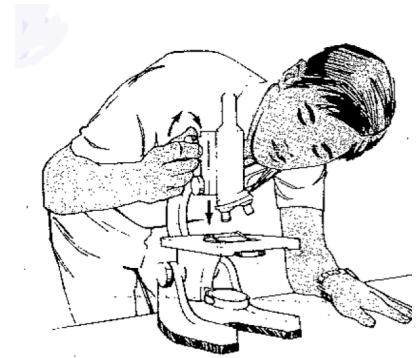
Part B. Locating and Position objects Under the Microscope

Procedure

- Click the low power objective into viewing position. NOTE: *Always locate an object first with low-power magnification even if a higher magnification is desired.*
- Adjust the diaphragm for the best light.
- Place a prepared slide of the letter “e” onto your microscope stage. Position the slide on the stage so the “e” faces you as it would on a magazine page. Position the letter “e” so that it is directly over the center of the stage opening. Secure the slide in place with the stage clips.

- Look to the side of your microscope as shown in figure 4. Slowly lower the low power objective by turning the coarse adjustment knob until the the knob cannot turn anymore. **CAUTION:** *Never lower the objective toward the stage while looking through the eyepiece.*
- While looking through the eyepiece with both eyes open, slowly turn the coarse adjustment knob so the letter “e” moves away from the objective lens.
- Bring the letter “e” into sharp focus by turning the the fine adjustment knob.

Figure 4



7. In plate P-1, draw a picture of the letter “e” as viewed through the microscope.

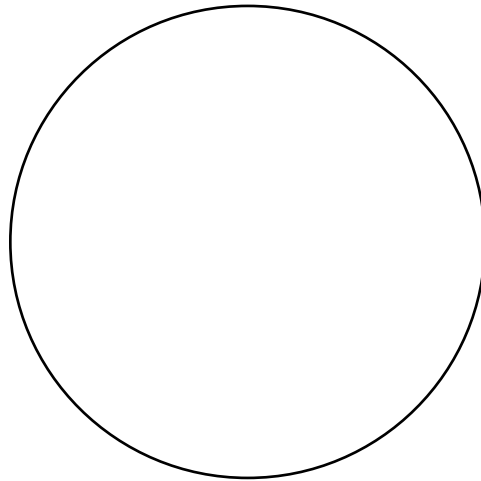


Plate P-1

Magnification _____

8. What is the position of the “e” viewed with the microscope compared to its position on the stage?

- While looking through the eyepiece, move the slide from *left* to *right*.

9. In what direction does the letter move as seen through the microscope? _____

- While looking through the eyepiece, move the slide from *right* to *left*.

10. In what direction does the letter move as seen through the microscope? _____

- While looking through the eyepiece, move the slide toward you.

11. In what direction does the letter move as seen through the microscope? _____

Part C. Increasing the Magnification of the Microscope

Procedure

- Any object to be viewed under high power magnification is *always located first under low power and focused*. Locate, center, and focus the letter “e” under low power.
- Move the low power objective out of viewing position. Look to the side of the microscope and revolve the nosepiece (Figure 5 & Figure 6). Click the medium power objective into viewing position. Locate, center, and focus the letter under medium power.

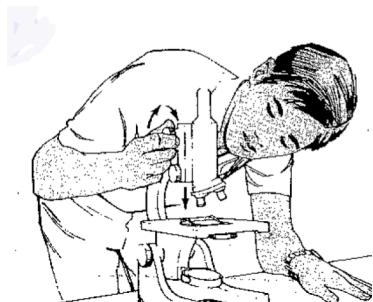


Figure 5

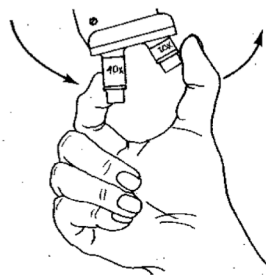
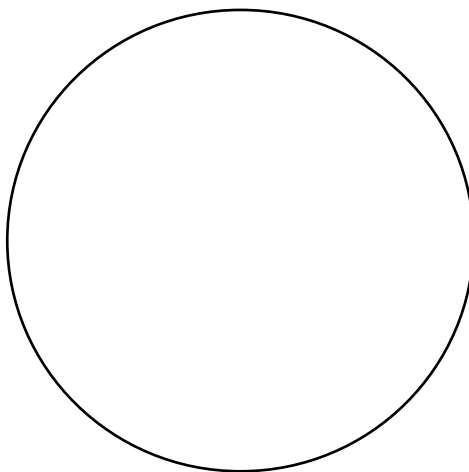


Figure 6

- Move the medium power objective out of viewing position. Look to the side of the microscope and revolve the nosepiece. Click the high power objective into viewing position. Look through the eyepiece. The letter “e” should be visible. Use the *fine adjustment knob* only to sharpen the focus. **CAUTION:** *never use the coarse adjustment knob for focusing with high power. Damage to the lens and slide may result if the coarse adjustment is used.*
- If you are unable to find the letter, then do the following. While looking through the eyepiece, move the glass slide slightly to the left, right, away from, or toward you. These movements may help reposition the letter directly in the center of the high power objective.
- Repeat Part C and D if you are unable to locate the object under high power.
- In Plate P-2, draw a picture of the letter “e” as seen with the high power lens.

Plate P-2
Magnification _____



Part D. Use of the Diaphragm

Procedure

- Place a wet mount slide of a few strands of absorbent cotton on the stage.
- Observe the cotton fibers with low power. While looking through the microscope, change the amount of light entering the microscope by adjusting the diaphragm.

12. Under what diaphragm setting (maximum, medium, or little light) are the cotton fibers sharpest?

- Change to high power and observe the cotton fibers. Remember to locate, center, and focus under medium power first. Again, readjust the amount of light entering the microscope.

13. Under what diaphragm setting (maximum, medium, or little light) are the cotton fibers sharpest?

All objects viewed under the microscope will require adjustment of light. Many problems associated with microscopic observation can be overcome by adjusting the diaphragm for proper lighting.

Part E. Depth of Field

Procedure

- Obtain the prepared slide labeled “Crossed Fibers”
- Locate the strands under low power. Center the slide so you are looking at the point where the strands cross. Adjust the diaphragm for proper lighting.

14. Can all strands be observed clearly at the same time under low power? _____

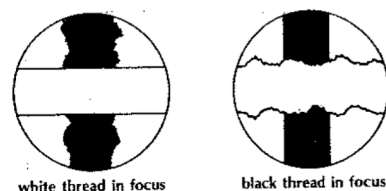
- Change to high power and observe the strands at the point where they cross. Remember to use medium power first.

15. Can all strands be observed clearly at the same time under high power? _____

The lens system of your microscope allows you to see clearly only one depth at a time under high power. In order to see objects at different depths, do the following:

- Turn the fine adjustment knob back and forth by a quarter of a turn while looking through the microscope. This movement will give a three-dimensional view of the object. Try this technique while looking at the crossed threads. Use figure 7 as a guide; note that first one strand is in front then the other.

Figure 7



- Look at the label on your slide. What “letter” slide do you have (A,B,C,D,E,or F)_____
- What is the order of the strands in your slide starting with the top strand._____

Part F. Stains as an Aid to Microscope Work

Procedure

Many objects observed with the microscope are colorless. Thus, they appear more transparent and are difficult to see. Stains often are used in microscope work to color objects for easier and more detailed observations. Stains can be added to a wet mount without disturbing the slide.

- Obtain a wet mount slide of unstained starch grains.
- View the wet mount slide with low power.
- In Plate P-3 draw several starch grains.
- Remove the slide from the microscope.
- Obtain a wet mount slide of stained starch grains.
- Observe the stained starch grains with low power.
- In Plate P-4 draw several starch grains.

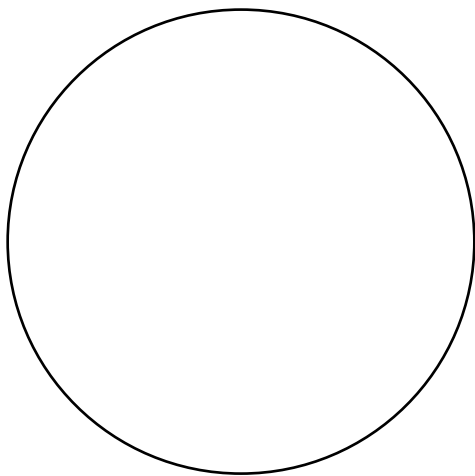


Plate P-3
Magnification_____

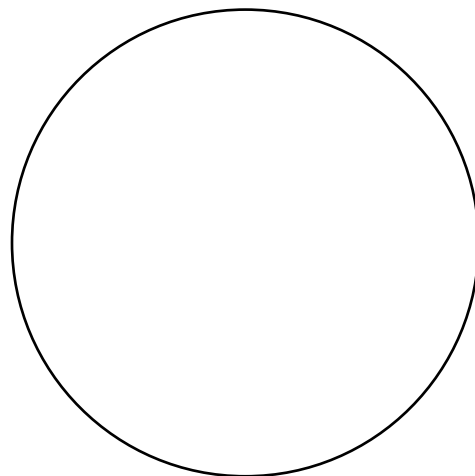


Plate P-4
Magnification_____

Part G. A Comparison of Fields of View

Procedure

Field of view is the area seen through a microscope. Is the field of view with low power greater than with high power, or are they the same? This exercise will help you answer this question.

- Move the slide to a less crowded area of starch grains. This area is usually near the outer edge of the coverslip.

- Examine the stained starch grains with low power.

16. Count and record the number of grains observed under low power. _____

- Without moving the slide, examine the starch grains with high power.

17. Count and record the number of grains observed under high power. _____

18. How does the number of grains observed under low power compare to the number under high power?

The width of your low power field is usually 10 times greater than that of your high power field. For example, if the low power objective has a magnification of 4X and high has a magnification of 40X, divide 40 by 4. Your answer (10) shows the difference in width of these two lenses. The low power objective has a width that is 10 times larger than that observed under high power.

19. Calculate the number of times greater the width of low power is than medium power for your microscope. Remember:

$$\frac{\text{Higher power objective}}{\text{Lower Power objective}} = \text{Number of times low power width is greater than high power width}$$

20. Calculate the number of times greater the width of medium power is than high power for your microscope.

21. Calculate the number of times greater the width of low power is than high power for your microscope.

Do not confuse width observed with total magnification. As magnification increases, width observed decreases.

22. Did you observe more or fewer starch grains under low power compared to high power?

23. Did you observe more or less area under low power compared to high power?

Analysis

1. Why must you always use the fine adjustment only when focusing with the high power?

Answer all of the following as true or false.

- _____ 2. Objects viewed under the microscope appear upside down.
- _____ 3. Total magnification of a microscope is determined by adding the eyepiece lens to the objective lens magnification.
- _____ 4. When moving the slide to the left, objects viewed through the microscope will move toward the left.
- _____ 5. An object should always be located first with low power.
- _____ 6. The diaphragm is used to adjust the amount of light entering the microscope.
- _____ 7. A light microscope should be carried in an upright position with both hands.
- _____ 8. All objects in different depths appear in focus at the same time while using high power.
- _____ 9. The fine wheel adjustment must be used to sharpen focus when using high power.
- _____ 10. Stains are used to help make clear objects appear darker under the microscope.
- _____ 11. Always look to the side of a light microscope when lowering the objective.
- _____ 12. Low power shows more area than high power.
- _____ 13. The eyepiece of a microscope is marked 10X. The high power objective is marked 50X. The total magnification is 500X.
- _____ 14. High power shows more detail than low power.
- _____ 15. Observers see about 10 times more width under low power than under high power.
- _____ 16. Your depth of field under high power is less than while observing under low power.